ROLE OF INVERTASE IN THE ACCUMULATION OF SUGARS IN COLD-STORED POTATOES

RUSSELL PRESSEY[1]

ABSTRACT

Freshly harvested potatoes contain low levels of total invertase and high levels of invertase inhibitor. Total invertase increases sharply when potatoes are placed in cold storage, but the inhibitor is not depleted in all varieties. The absence of basal invertase activity in Norchip tubers indicates that an excess of invertase is not required for reducing sugar formation. Analyses of 37 varieties and seedlings after 3 months cold storage revealed that the reducing sugar content is not proportional to the invertase activity. High sugar contents are associated with low inhibitor levels, but low sugars are not necessarily associated with high inhibitor levels. The results indicate that invertase participates in reducing sugar formation, but other factors are responsible for the regulation of starch-sugar conversion in potatoes during cold storage.

RESUMEN

Papas acabadas de cosechar contienen niveles bajos de invertasa total y niveles altos de invertasa inhibidora. Invertasa total incrementa rapidamente cunado las papas se almacenan al frío, pero el inhibidor no desaparece en todas las variedades. La ausencia de actividad basal de invertasa en tubérculos Norchip indica que un exceso de invertasa no es requerido papa la formación de azúcar reductor. El análisis de 37 variedades y brotes después de 3 meses de almacenamiento al frío reveló que el contenido en azúcar reductor no es proporcional a la actividad de invertasa. Altos contenidos en azúcar están asociados con niveles bajos de inhibidor, pero azúcares bajos no están necesariamente asociados con niveles bajos del inhibidor. Los resultados indican que la invertasa toma parte en la formación de azúcar reductor, pero otras factores son responsables por la regulación de la conversión de almidón a azúcar en las papas durante el almacenamiento.

The sweetening of potatoes stored at low temperatures is an important problem in potato processing. Sucrose, glucose, and fructose are the major sugars that accumulate, but the levels of the reducing sugars, glucose and fructose, determine the suitability of potatoes for chipping. Successful methods for the prevention of sugar accumulation have not been devised, and it is still necessary to condition cold-stored potatoes at a warm temperature to reduce the sugars to an acceptable level.

A prerequisite for blocking sugar accumulation chemically is elucidation of the biochemical pathway of starch-sugar transformation. This

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pathway remains unknown, but the results of recent studies indicate that a sucrose intermediate is involved in glucose and fructose formation. Sucrose increases most rapidly when potatoes are first exposed to low temperature, but glucose and fructose are usually the predominant sugars after prolonged cold storage (6, 7). Furthermore, glucose and fructose are often present at about equimolar amounts (6), suggesting that they arise by invertase-catalyzed hydrolysis of sucrose. Direct evidence for a role of invertase in sugar accumulation has been presented during the last few years (2, 3, 4, 5, 7).

This paper presents additional information on invertase in potatoes. The results are in accord with a role for invertase in sugar accumulation but suggest that other factors are important in the regulation of the overall conversion of starch to sugars.

Materials and Methods

The potatoes used in this study were grown on the research farm of the Red River Valley Potato Growers' Association, Grand Forks, N. Dak. in 1966 and 1967. The tubers were harvested when they appeared to be mature and were cured at 65 F (18.3 C) for 10 days before subjecting them to the various storage conditions.

Invertase, invertase inhibitor, and sugars were measured as described earlier (4). Only the free or excess inhibitor is determined by the procedure for invertase inhibitor.

Results

Changes in Composition at 40 F (4.44 C) — The results for 1966 Kennebec potatoes are presented in Fig. 1. Total invertase and reducing sugars were low while invertase inhibitor was high at harvest time. Both total invertase and reducing sugars increased sharply after the tubers were placed in cold storage. Invertase increased until it exceeded the invertase inhibitor and basal invertase activity appeared. The basal invertase activity persisted during the period of sugar accumulation, but it decreased and was replaced by an excess of invertase inhibitor after the sugars reached their maximum level. These changes are similar to those described earlier for the variety Pontiac (4).

A feature not observed previously is the reappearance of basal invertase after prolonged cold storage. Total invertase began to increase after 24 weeks cold storage and continued to increase rather rapidly. After 60 weeks, the basal activity was 14.5 units/mg protein and total invertase was 19 units/mg protein. Despite the high invertase activity, the sugars remained low (1.05%).

The variety Norchip contained more than twice as much invertase inhibitor as Kennebec at harvest (Fig. 2). The level of invertase inhibitor decreased markedly during cold storage of Norchip potatoes, but it was not depleted. Invertase increased during cold storage but to a maximum lower than in Kennebec. Despite the absence of basal invertase activity, the tubers accumulated a moderate level of reducing sugars. Invertase did not increase again on very long cold storage as it did in Kennebec tubers.

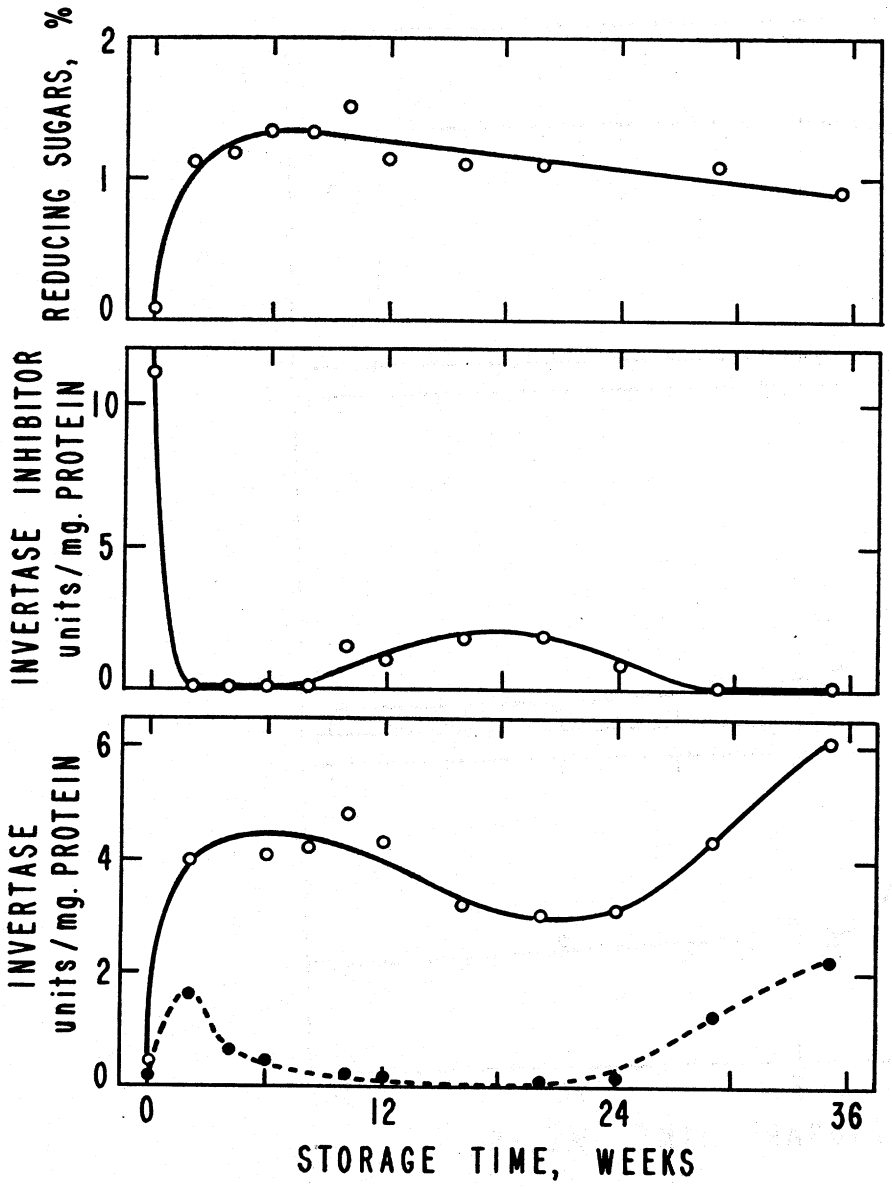

FIG. 1.—Changes in composition of Kennebec potatoes stored at 40 F. •---• = basal invertase; ⊙——⊙ = total invertase.

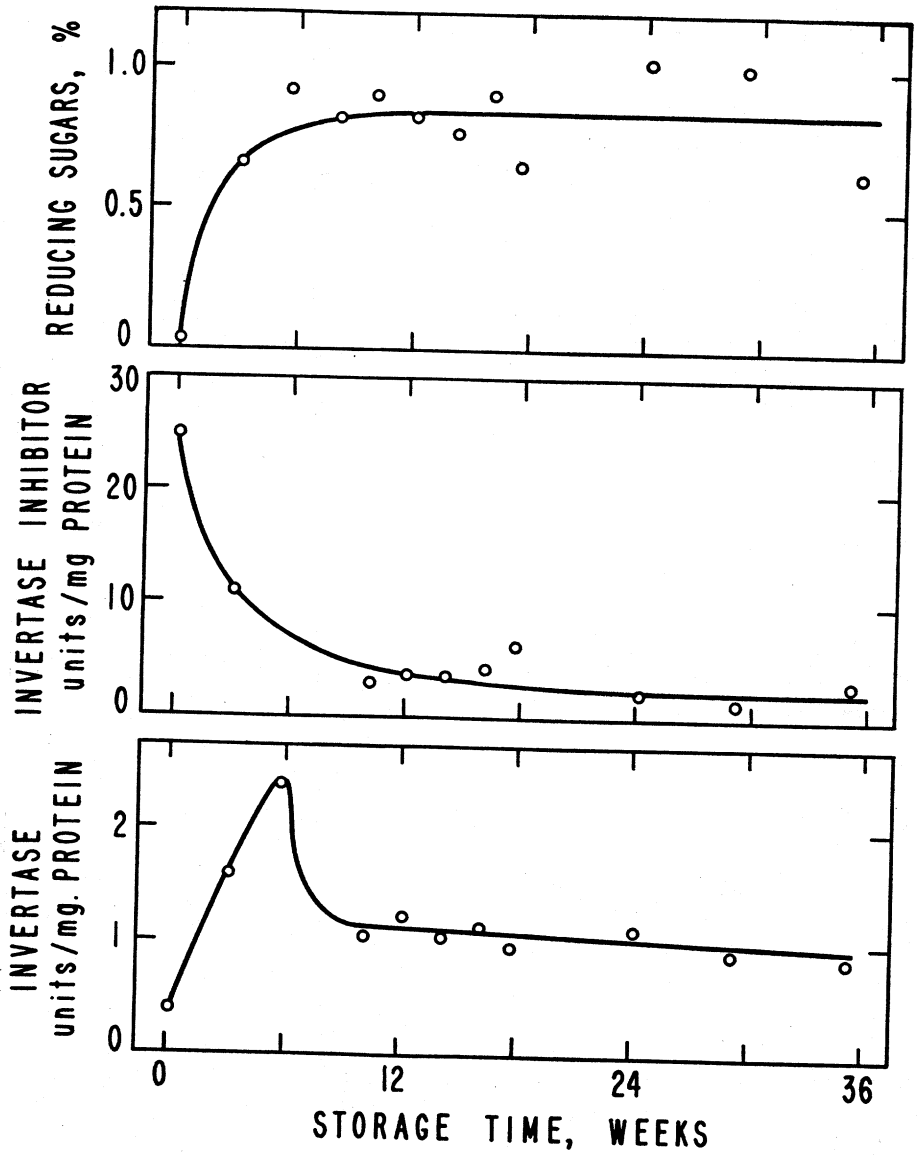

FIG. 2.—Changes in composition of Norchip potatoes stored at 40 F.

Changes in Composition at 95 F (35 C). — Potatoes accumulate soluble sugars at high as well as low storage temperatures. Both effects are stresses on the tubers, but it is not known if the responses involve similar mechanisms. When Kennebec tubers were stored at 95 F, sucrose was the main sugar that accumulated, and glucose and fructose were

present at negligible levels. Invertase did not develop, and while the excess invertase inhibitor decreased, it did not disappear.

Varietal Differences in Composition. — The amounts of sugars that different varieties of potatoes accumulate are highly variable. It is important to establish whether the levels of invertase and its inhibitor are related to the varietal differences in sugars. A problem in such a study is the selection of a sampling time because of the dynamic nature of the system. Time studies would have to be limited to a few varieties.

In one study, potatoes were sampled after 3 months at 40 F (4.44 C) which correspondents to an approximately steady state in Kennebec, Norchip, and Pontiac. Thirty-seven varieties and seedlings of the 1967 crop were analyzed over a 3-week period (Fig. 3). It is obvious that invertase activity is not related to the reducing sugar content. The variety Superior had basal invertase and high total invertase activity but only moderate reducing sugars. All potatoes with sugars higher than 1% contained low levels of invertase inhibitor. On the other hand, only some of the potaoes with sugars lower than 1% had high levels of invertase inhibitor.

Discussion

Numerous attempts have been made to identify the enzymes in the starch-sugar transformation in potatoes (1, 2, 3, 4, 7). The most common approach is to correlate enzyme activities with sugar levels. One weakness in this method is that it is unlikely that all of the enzymes in this pathway are activated for the relatively slow process of sugar accumulation. Only enzymes specifically involved with sugar accumulation could be identified by this method. Furthermore,, if an enzyme increases during the period of sugar accumulation, it may decrease after the sugars have reached their maximum level. Regular and frequent sampling of tubers is therefore necessary to correlate compositional factors with sugar formation.

The data presented here indicate that invertase is one of the enzymes associated with the formation of reducing sugars. This enzyme is present at very low levels in freshly harvested tubers, and its activity is suppressed by an excess of a specific, proteineous inhibitor. When potatoes are exposed to low temperatures, total invertase increases sharply, presumably by synthesis of new enzyme. In the variety Kennebec, invertase formation continues until it exceeds the level of inhibitor and basal invertase activity develops. After the sugars stop increasing, invertase decreases to the point where basal invertase activity is replaced by an excess of invertase inhibitor.

The changes in composition in tubers stored at high temperatures support the view that invertase is required for the formation of reducing sugars. Under these conditions, sucrose is the only sugar that accumulates. Hydrolysis of sucrose does not occur because invertase activity does not develop.

On the other hand, the reappearance of basal invertase activity after prolonged cold storage of Kennebec potatoes is not accompanied by a corresponding increase in sugars. The renewed synthesis of invertase may be a symptom of senescence. The reason why sugars do not accumulate, despite basal invertase activity, may be that the substrate sucrose

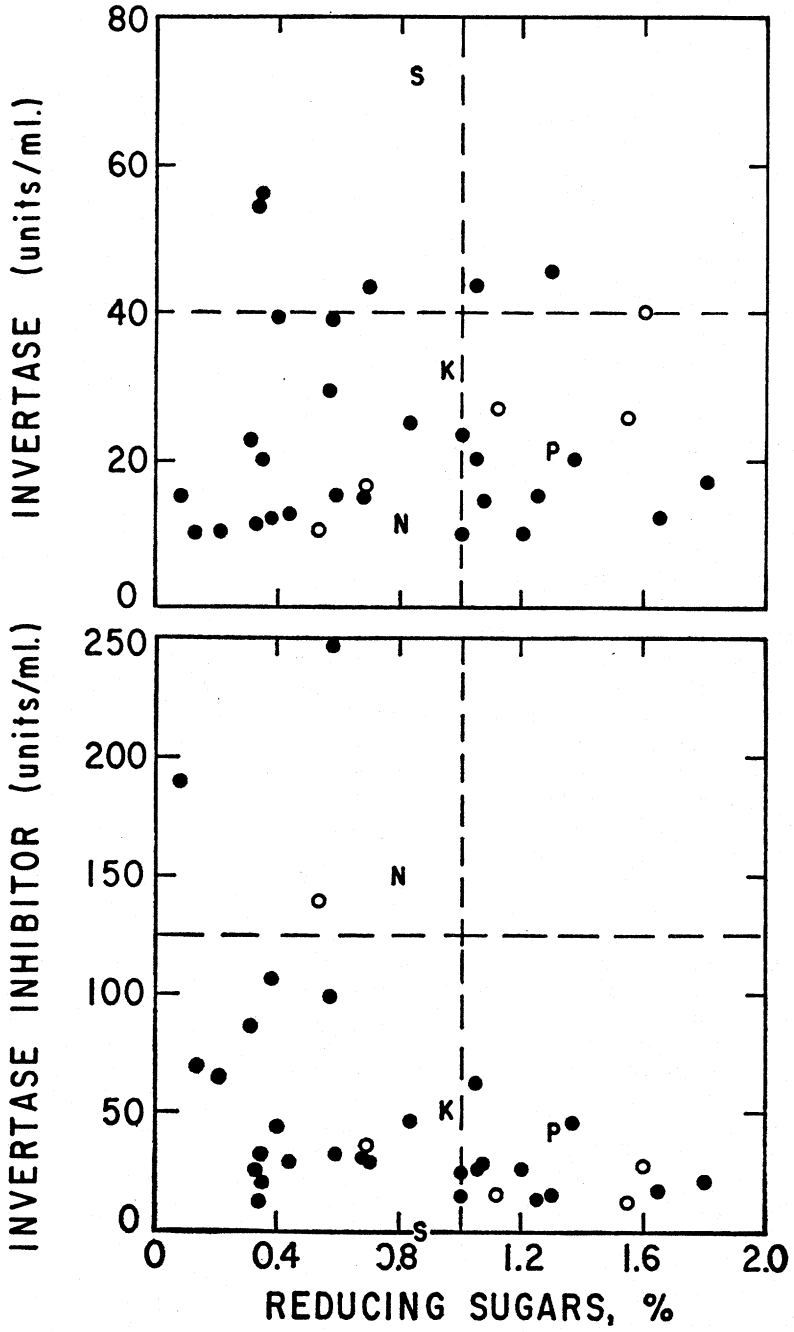

FIG. 3.—Plots of invertase and invertase inhibitor against reducing sugar contents. K = Kennebec; N = Norchip; P = Pontiac; S = Superior; o = seedlings provided by Dr. R. Johansen, North Dakota State University, Fargo; • = seedlings provided by Dr. F. Lauer, University of Minnesota, St. Paul.

is not available to the enzyme. It is clear that other factors regulate sugar formation.

The results of studies on the variety Norchip are not consistent with the theory that basal invertase is required for reducing sugar accumulation. This variety accumulated moderate levels of glucose and fructose in the absence of basal invertase activity. Nevertheless, invertase did increase and the excess inhibitor was reduced to a very low level. It may be that the invertase activity in a tuber extract does not represent the actual situation at the enzyme site within the cell. Thus, invertase may function in the presence of an excess of inhibitor if the two are separated within the cell.

Analyses of a large number of varieties and seedlings show that the sugar contents are not related to the levels of invertase and invertase inhibitor in the potatoes after 3 months of cold storage. This does not necessarily mean that invertase does not participate in reducing sugar formation. All of the samples contained significant levels of invertase and therefore it was available for the hydrolysis of sucrose. Invertase may be one of the enzymes in the starch-sugar conversion, but the regulation of the overall transformation is not related to the level of invertase.

Sucrose is not only an intermediate in the formation of glucose and fructose but also a major product of starch degradation. Unless glucose and fructose exert feedback inhibition (8), the regulation of sucrose formation is independent of invertase. This leaves the hydrolysis of sucrose as the only function of invertase, and the availability of substrate for its action depends on a separately controlled process. Nevertheless, changes in activity with changes in storage temperature and the presence of the inhibitor in potatoes are evidence that the invertase step is highly regulated.

Acknowledgment

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